Interaction of Erythrocyte Membranes with Particulates

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Chrysotile fibers promoted the fusion of human erythrocyte membranes and the hemolysis and fusion of fowl erythrocytes. Amosite and glass fibers did not show the same effect. These findings provide a model for the internalization of the fibers by animal cells.

Introduction

The cytotoxic properties of asbestos fibers have been shown to be correlated with their physical and structural nature (1, 2), as well as with their ability to induce mesotheliomas when injected into animals (3). The relative cytotoxicity of the different forms of asbestos (chrysotile, crocidolite and amosite) is the subject of differences of opinion with different forms being used in different countries depending on availability.

The interaction of these fibers with biologic or model systems has been extensively studied. Early investigations indicated that chrysotile interacts with cell membranes causing hemolysis of erythrocytes or with macrophages causing the release of lactate dehydrogenase (4). Internalization of all three types of fibers by human foreskin fibroblasts (5) and macrophages (6) has been reported. Beck (7) has reported that chrysotile could bring about the fusion of human fibroblasts in culture.

The present report focuses on the membrane fusion-promoting properties of chysotile with erythrocyte ghosts and chicken erythrocytes as model systems.

Materials and Methods

Erythrocyte Ghosts

Unsealed human erythrocyte ghosts were prepared by osmolarity and pH manipulation according to the method of Dodge et al. (8), as modified by Ottolenghi and Bowman (9).

Chicken Erythrocytes

White Leghorn roosters were bled and the blood collected into Alsevier's anticoagulant solution (1/3 v/v). The cells were washed three times in 0.85% NaCl and a final 1% working suspension made in this same diluent.

Particulates

Short NIEHS chrysotile (mean length and width of 21 and 0.17 μm, respectively), NIEHS amosite and Pyrex glass fibers were suspended in 20 mOSM sodium phosphate buffer, pH 7.8, to a final concentration of 0.1 mg/mL. The suspensions were stored at 4°C and vigorously shaken before use.

Chemicals

All chemicals used were reagent grade. Polymyxin B was obtained through the courtesy of Pfizer Inc., Brooklyn, NY.

Incubation of Erythrocyte Ghosts with Particulates

Erythrocyte ghosts (1.5 × 10⁷) in 0.05 mL 20 mOSM sodium phosphate buffer, pH 7.8, were

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mixed with 5µg of particulate in 0.05 mL of the same buffer, and then the mixture was diluted to a final concentration of 2 mOSM with distilled water. Controls consisted of ghost suspensions diluted but without addition of particulates or, of the entire system maintained in 10 mOSM buffer.

Polymyxin B (final concentration 5 µg/mL) was added to the mixtures prior to dilution with distilled water.

The mixtures were incubated in a water bath at 25°C, and wet mounts were examined periodically by dark field or phase contrast microscopy.

Incubation of Chicken Erythrocytes with Particulates

A mixture of 0.05 mL of a 1% suspension of chicken erythrocytes in 0.15 M NaCl and 5 µg of particulate in 20 mOSM buffer was diluted (1/10 v/v) with 0.15 M NaCl and incubated at 25°C. Samples were withdrawn at appropriate intervals and examined by phase contrast microscopy.

Results

When human red cell ghosts were incubated with chrysotile in 2 mOSM buffer, the fusion seen in Figure 1 was obtained. When the incubation medium had an osmolarity of 20 mOSM, binding but no fusion was observed (Fig. 2a).

Under conditions where polymyxin B was added to the incubation mixture, binding was observed to occur localized at sites showing enhanced refractivity. No fusion was seen in any of the polymyxin-containing preparations (Fig. 2b).

Incubation of the chicken erythrocytes with chrysotile resulted in binding, hemolysis and occasional fusion, whereas when amosite or glass fibers were used, neither binding, fusion nor lysis were observed (Fig. 3 a, b, d).

Discussion

The incubation of asbestos with erythrocytes has been observed to result in hemolysis of the cells (10-12). Chrysotile has also been reported to bind to erythrocyte ghosts (10), but there are no reports in the literature concerning the facilitation of membrane fusion by chrysotile fibers.

Our system is particularly suited for this purpose, since the exposure of erythrocyte ghosts to a 2 mOSM environment will remove the spectrin network and allow for greater plasticity of the membrane. These conditions have been used to prepare red cell membrane vesicles (13). When the temperature is kept around 25°C, the ghost membrane becomes more plastic but does not break up as readily
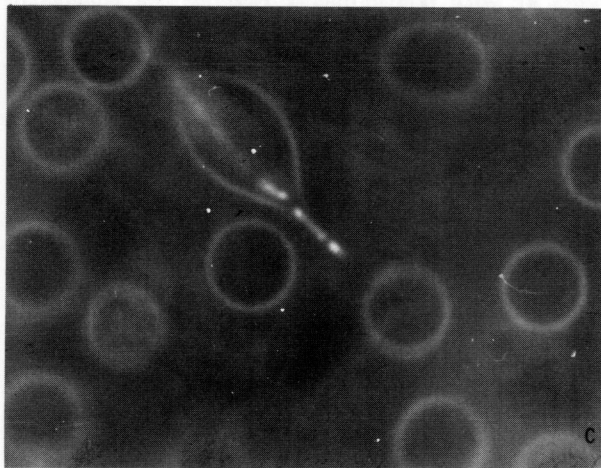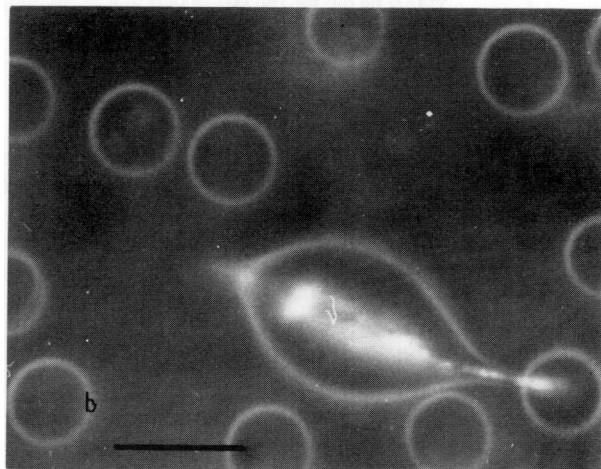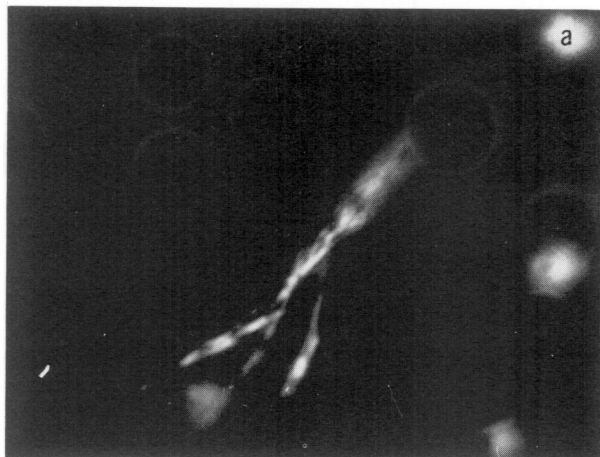

FIGURE 1. Dark field micrographs of human red cell ghosts incubated with chrysotile at 25°C in 2 mOSM phosphate buffer: (a) fused membranes across branched fiber; (b, c) fused membranes around mostly linear fiber. Bar = 10 mm
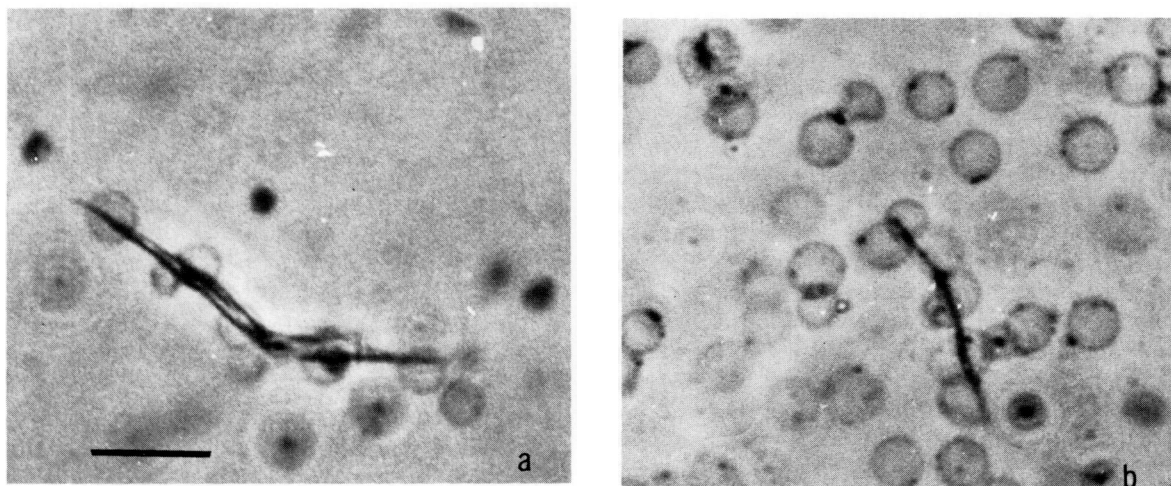
FIGURE 2. Phase contrast micrographs of human red cell ghosts: (a) incubated with chrysotile at 25°C in 20 mOSM phosphate buffer; (b) incubated with chrysotile and polymyxin B (5 μg/mL) in 2 mOSM phosphate buffer at 25°C. Bar = 20 μm.
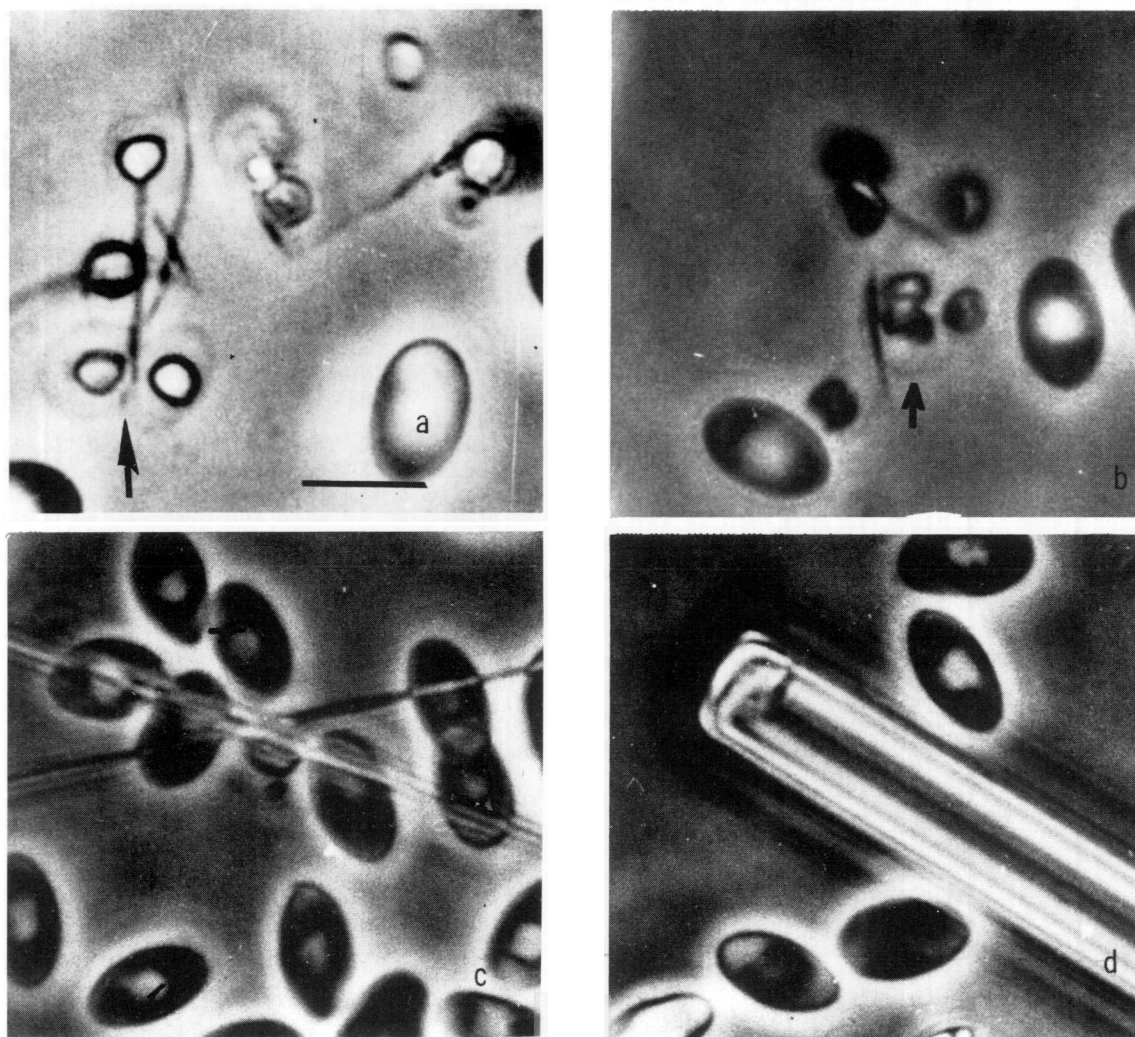

FIGURE 3. Phase contrast micrographs of chicken red cell incubated with (a, b) chrysotile or with (c) lamosite or (d) pyrex glass fiber in 0.85% NaCl. Arrows indicate lysed cell (a) and lysed and possibly fused cells (b). Bar = 20 μm.

into vesicles. In other experiments with this model system, it was seen that the addition of polymyxin B (as well as some other antibiotics) and Ca^{2+}, also inhibited the formation of membrane vesicles.

Magnesium ion, together with Ca^{2+}, has been implicated as part of the process of fusion on phospholipid liposomes as well as of the fusion of cellular membranes (14). It is postulated that the Mg^{2+} serves as a bridge between phosphate sites on membranes and that a sequence of conformation changes brings about fusion of the apposite leaflets. This process requires a certain level of membrane fluidity which can be modulated by varying the temperature or salt concentration.

Since chrysotile is coated with magnesium on which one of the positive charges is free, it is not surprising that the fiber will bind phospholipids and membranes; indeed, this had been postulated by McNab and Harington (15) and reported by Jaurand et al. (10). The fusogenic properties of the fiber are not surprising, since it can be envisioned that conformational changes will occur related to the binding of the membrane to the magnesium.

The high refractivity sites observed on ghosts treated with polymyxin can be related to the cooperative association of this antibiotic with the membrane (16) which is postulated to result in a small area of exposed phosphate groups surrounded by a layer of polymyxin-covered phospholipid. It is thus possible to postulate that such an exposed area would bind the fiber but that in the absence of sufficient neighboring phosphates there may not be adequate apposition to produce fusion.

Internalization of asbestos into macrophages and fibroblasts has been demonstrated by several authors and is described in a companion report from our laboratories (17). From our own data as well as those of other authors, we have not been able to discern consistently clear membrane-bound vacuoles around the asbestos. It would appear possible that the internalization occurs by the membrane enveloping the fiber along its long axis (6) followed by fusion, just as in phagocytosis, but since the membrane would be tightly bound to the fiber through the array of magnesium ions, no phagocytic vacuole would be discernible.

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